Review of Literature
3. REVIEW OF LITERATURE:

3.1 Introduction to plant: *Gmelina arborea* Roxb.

3.1.1 Introduction to family: Verbenaceae

Verbenaceae family includes herbs, shrubs, or trees. Leaves usually opposite or whorled, simple or (in *Vitex*) digitate; stipules 0. Inflorescence cymose, racemose or spicate; cymes often compound or paniculate; bracts usually small; flowers often brightly coloured, hermaphrodite (rarely polygamous), usually irregular. Calyx inferior, gamosepalous, persistent, tubular or cup-shaped, 5-4 (rarely 6-8) lobed or toothed. Corolla gamopetalous; tube usually cylindrically or dilated above, often curved; limb 2- lipped or sub equally lobed; lobes 5-4 (rarely more). Stamens 4, didynamous (rarely 2, very rarely 5-6), inserted on the corolla-tube; filaments free; anthers 2-celled, opening by longitudinal slits. Disk usually inconspicuous. Ovary superior, sessile 2-4 (rarely 8- or almost 1-) celled, entire or 4-lobed; ovules variously attached, 2 (sometimes 1) in each cell; style terminal; stigma usually entire, less commonly 2- or more lobed. Fruit usually more or less drupaceous, 2-4 or 1-celled; mesocarp juicy, fleshy or dry; endocarp usually bony. Seed erect or pendulous, separate in distinct cells; embryo straight; radicle inferior. Genera about 70. Species 750. Almost all tropical and sub-tropical. The few members, who are endowed with medicinal properties, are mostly bitter and more or less astringent (Kirtikar and Basu, 1999).

3.1.2 Introduction to genus: *Gmelina* Linn.

It includes trees and shrubs distributed chiefly in south-east Asia and tropical Australia (The Wealth of India, 1956).

The genus *Gmelina* belongs to the subfamily Viticoideae, of the Verbenaceae and is a sub cosmopolitan genus centered in the warm temperate regions of the Mediterranean and South Asia (Hosny and Rosazza, 1998).

Trees or shrubs unarmed or spinous; young shoots usually tomentose. Leaves opposite, entire or toothed, sometimes more or less lobed. Flowers large, yellow or brownish yellow, often tomentose, in small dense or lax sessile or pedunculate cymes along the branches of a terminal panicle; bracts usually narrow, rarely leafy. Calyx campanulate, 4-5 toothed or sub entire, persistent
and unaltered in fruit. Corolla 2-lipped, infundibuliform, ventricose in the upper part; tub slender below, much swollen above; limb oblique, spreading, 4-5 lobed. Stamens 4, didynamous, inserted below the throat, shorter than the corolla; anthers with oblong more or less discrete cells. Ovary 4-celled; ovule solitary in each cell; style slender; stigma shortly 2-fid. Fruit, a succulent drupe; endocarp undivided, bony 2-4 celled. Seeds oblong albumen 0; cotyledons thick. Species 8. Indo-Malaya, Australia. G. arborea, Roxb. is likewise an East Indian tree, the root of which has been employed in gout, and the bark in intermittent fevers. The smaller species Gmelina parvifolia, Roxb. and G. asiatica, Lin. possess demulcent properties, the leaves and the root being employed. Three species occurs in India (John, 1885; WOI, 1956).

2. A shrub......... Gmelina asiatica Linn.
3. A shrub......... Gmelina elliptica Sm. Syn. Gmelina villosa Roxb

G. arborea Roxb. Hort. Beng.:

It is commonly known as Gambhari, Shriparni or sewan. It is an unarmed handsome tree, found scattered in deciduous forests through out the greater part of India and Andaman’s. Bark smooth, whitish grey; leaves opposite, broadly ovate; flowers in terminal panicles; drupe fleshy, 1 to 2 seeded. Roots are used as bitter tonic, stomachic, laxative and galactagogue.

G. asiatica Linn.

It is commonly known as Gopabhadra or nagphul. It is a large straggling, spinescent shrub, sometimes climbing, distributed in the Deccan Peninsula. Leaves small, ovate; flowers yellow in terminal tomentose racemes; drupes ovoid and 2 seeded. Roots are used in gonorrhea, catarrh of the bladder, rheumatism and as a blood purifier.

G. elliptica Sm. Syn. G. villosa Roxb:

It is a shrub or a small tree found in Nicobar Islands. Leaves ovate-elliptic; flowers yellow, small; drupes ovoid or obovoid with 1 to 2 seeds. This plant is used in poultices for headache and swelling.

It is a thorny shrub grown in gardens for its large yellow flowers and conspicuous brightly coloured bracts. In Malaya, the plant is pounded with lime and applied as a poultice to the throat for relieving cough.


(WOI, 1956; Srivastav et al., 1989; Rastogi et al., 1990; Chopra et al., 1999; Kirtikar and Basu, 1999).

Synonyms:  
Gmelina indica Burm. f.  
Gmelina rheedii  
Premna arborea

Vernacular Names:

Sans:--- Gambhari; Krishna Vrinlaka; Shriparni; Kasmari.  
Hindi:-- Gambhara; Kambhar; Gumbhar; Kambari.  
Ben:---- Gamari; Gaenari; Gumar; Gumbar, Joogani, Chookur.  
Guj:---- Shewan.  
Punj:--- Kumhar; Gumar.  
Tam:--- Gmuadu-teku; Gumadi, Tagvomooda.  
English: Snap Dragon Tree, Coomb Tree, Candhar Tree, Malay bush-beech, white beech, white tea

Scientific classification

Kingdom: Plantae – Plants  
Subkingdom: Tracheobionta – Vascular plants  
Super division: Spermatophyta – Seed plants  
Division: Magnoliophyta – Flowering plants  
Class: Magnoliopsida – Dicotyledons  
Subclass: Asteridae  
Order: Lamiales  
Family: Verbenaceae – Verbena family  
Genus: Gmelina L. – Gmelina  
Species: Gmelina arborea Roxb. – Gumhar
Distribution:
Throughout India, Ceylon, Malayan and Philippine Islands. In India, *G. arborea* occurs extensively in sub-himalayan tracts, common throughout Assam and adjoining areas of Northern West Bengal, also in South Bihar and Orissa, sporadically found in western and southern India and planted elsewhere on a large scale. It also occurs naturally in Myanmar, Thailand, Laos, Cambodia, Vietnam and in southern provinces of China, but planted extensively in Sierra Leone, Nigeria, Malaysia and on experimental basis in other countries as well.

Plant Description: (WOI, 1956; Srivastav et al., 1989; Rastogi et al., 1990; Chopra et al., 1999; Kirtikar and Basu, 1999; Kapoor, 2005).
A moderate sized unarmed deciduous tree, with branchlets yellowish-tomentose, reaching 18 m/60 ft high, with a clear bole of 20-30 ft. and girth of 5-7 ft., found scattered in deciduous forests throughout the greater part of India.
and the Andaman’s, up to an altitude of 5,000 ft.; it is also cultivated in gardens and avenues.

Bark grayish yellow, rather corky; branch lets and young parts clothed with fine white mealy pubescence.

Leaves opposite, petiolate, the petioles 5-15 cm. long, puberulent to glabrous, the leaf blades broadly ovate, 10-25 x 7.5-18 cm., base cordate or sometimes truncate and shortly cuneate, long-acuminate at apex, entire at margin (but sometimes toothed or lobed on young plants), tomentose or at length glabrous above, densely and persistently tomentellous beneath with stellate hairs, glanduliferous just above petiole, the lateral nerves 5-10 per side (Smith, 1991).

Flowers appearing with or sometimes before the young leaves, inflorescences fulvous-tomentose throughout, usually in small cymes of about 3 flowers arranged along the branches of densely fulvous-hairy panicle reaching 30 cm long; buds clavate, angular; bracts 8 mm. Long, linear-lanceolate. Calyx 5 mm. Long, broadly campanulate, densely fulvous hairy; teeth 5, small, triangular, acute, corolla showy, yellow to reddish or brownish, 25-40 mm. long, the tube densely pubescent without; stamens 4, exerted, one pair sometimes sterile, the bracts linear to linear-lanceolate.

Fruit is drupe longer and broader than the obovate rounded lateral lobes. Drupe 2-2.5 cm. Long, ovoid or pyriform, smooth, orange-yellow when mature, aromatic, the endocarp usually 2-celled and 2-seeded (or by abortion 1-seeded), or sometimes 3-celled and 3 seeded.

Roots occurs in pieces with secondary and tertiary branches, root pieces nearly cylindrical with uneven surface, grayish brown, fracture somewhat tough in bark, brittle and predominant in woody portion. Mature root bark when fresh, yellowish in colour; dry pieces curved and channeled, thinner ones forming single quills, external surface rugged due to presence of vertical cracks, ridges, fissures and numerous lenticels; fracture short and granular; taste, mucilaginous, sweetish with slight bitterness.
Uses:

The drapes, leaves, flowers, roots and bark are used in traditional system of medicine.

The root is a bitter tonic, stomachic and laxative. In the form of infusion or decoction it is prescribed in indigestion, fevers and anasarca. It is given as a galactogogue with liquorice, sugar and honey added, in cases of scanty secretion of milk in women. It is also used as appetite stimulant and in the treatment of liver disorders (Deka et al., 1983; Rastogi et al., 1990; Sharma and Balakrishna, 1993; Chopra et al., 1999).

Pulverized root is applied locally for gout. Roots of *Gmelina* are used as an ingredient in many well-known Ayurvedic preparations like Dashmula and Chyawanprasha and is therefore much used in a variety of diseases (Tewari, 1995). The root constitutes one of the *moola* in Dashmool and also Panchmool drug of Ayurveda. It belongs to group of five major roots (Mahat panchmula) of Dashmula di kvatha. The main preparations are Shripamaadi kwaatha and Panchmulaadi kwaatha. This group is specific for chronic fever, rheumatic affection, hemorrhages, urinary tract infection, anuria and dysuria. Roots are useful in hallucinations and also as an anthelmintic. It is also valued for its use in treatment of piles, abdominal pains, burning sensations, and urinary discharges (Rastogi et al., 1990; Sarin, 1996; Chopra et al., 1999).

The plant is recommended in combination with other drugs for the treatment of snakebite and scorpion-sting. In snake-bite, a decoction of the roots and bark (1 in 16) is given internally (WOI, 1956; Kirtikar and Basu, 1999).

To prevent abortions in the early stage of pregnancy a powder of the bark with manjista and satavari is given in milk. The bark is bitter tonic and stomachic, and is considered useful in fever and indigestion. The flowers are sweet, cooling, bitter, acrid; astringent; useful in leprosy and blood diseases. Fruit forms an ingredient of several cooling and refrigerant decoctions. The fruit is acrid, sweet; cooling, diuretic, tonic, aphrodisiac, alterative, astringent to the bowels; it promotes the growth of hair; also used in anemia, leprosy and ulcers (WOI, 1956; Rastogi et al., 1990; Warman, 1991; Nadkarni, 1998; Chopra et al., 1999; Khory and Katrak, 1999; Kirtikar and Basu, 1999; Ayurvedic Pharmacopoeia of India (API) 2004).
The juice of the leaves is used to remove foetid discharges and also as a demulcent in gonorrhea and cough, when given with sugar candy. Leaves ground into paste with water are applied to the forehead for headache in fevers. The leaf juice is used as a wash for foul ulcers (Khory and Katrak, 1998; Nadkarni, 1998).

The wood of the tree is strong and durable. It is used for making furniture, paneling, musical instrument, artificial limbs, agricultural implements, matchsticks, matchboxes etc. The wood ash and the fruits are used for dyeing (Warman, 1991).
3.2 Review of literature of plant:

3.2.1 Pharmacological reviews:

Anti-viral Activity:
Aqueous extract (ethanol: water, 1:1) of stem bark at the concentration 50µg/ml exhibited weak antiviral activity against cell culture of Ranikhet virus and had no cytotoxic activity against cell culture CA-9KB (ED₅₀ > 20µg/ml) (Dhar et al., 1968). Further, the ethanolic extract of dried stem bark was also reported to possess antiviral activity against cell culture of virus Ranikhet (Kaij et al., 1992).

Hypoglycemic activity:
Alcoholic extract of stem bark and wood were found to possess hypoglycemic activity in albino rats with dose of 250mg/kg. In quantitative toxicity assessment, aqueous extract showed maximum tolerated dose (1gm/kg) when administered intra peritoneal in mouse (Dhar et al., 1968).

Anti-malarial activity:
The ethanolic extract of dried stem exhibited antimalarial activity against *plasmodium falsiperum* (IC₅₀ = 36µg/ml), while ethanolic extract of roots (IC₅₀ = 85µg/ml) and root bark (IC₅₀ = 88µg/ml) showed weak anti-malarial activity against *plasmodium falsiperum* in the same set of experiment (Simonsen et al., 2001).

Nematocidal Activity:
The wood decoction was reported to show weak nematocidal activity against *Toxocara canis* when tested at concentration 10mg/ml (Kiuchi et al., 1989).

Cardio-vascular activity:
The hypolipidemic activity of multi component formulation, containing *G. arborea* as one of the ingredient was reported. With intragastric administration (50mg/kg) in rats, this was found active in lowering serum β-lipoprotein and apoprotein levels (Khanna et al., 1991).

Different extracts of shade dried roots of *G. arborea* were evaluated for potential angiotensin converting enzyme inhibitory activity. Acetone and ethanolic extract at a concentration 0.33mg/ml exhibited weak inhibitory
activity, while aqueous extract at the same concentration found inactive (Somnadhan et al., 1999).

**Anti-inflammatory activity:**
50% ethanolic extract of dried stem bark exhibited significant anti-inflammatory activity, when given intraperitoneally at a dose 500mg/kg to rats of both sex (Agrawal et al., 1994).

**Anti-fungal Activity:**
Heartwood of the Malaysian *G. arborea* species was investigated for antifungal activity against *Trametes versicolor* and *Fomitopsis palustris*. Ethyl acetate soluble fractions were found rich in lignans and showed the highest activity against both fungi species. The effect was thought to be contributed by the piperonyl nucleus of lignans and synergism by coexistence of five compounds (Kawamura and Ohara, 2004).

**Miscellaneous:**
The effect of bark and fruit aqueous extracts on parquet and hydrogen peroxide induced oxidative stress was investigated using liver slice culture. Both parquet and hydrogen peroxide were found to be cytotoxic as measured by release of lactate dehydrogenase from liver slice culture. Addition of bark and fruit extracts along with these cytotoxic agents led to a decrease in lactate dehydrogenase release. Activities of three antioxidant enzymes, namely superoxide dismutase, catalase, and glutathione reductase, were found to increase on treatment with these pro-oxidants. Addition of the plant extracts along with the pro-oxidants suppressed the enzyme activities. The extracts also displayed antioxidant activity in *in vitro* radical scavenging assays. Results indicated that *Gmelina* bark and fruit extracts protected liver slice culture cells by alleviating oxidative stress–induced damage to liver cells (Sinha et al., 2006).
The effect of fruits was studied in rabbits by considering electrophoratic analysis of serum fractions, body weight and physical behavior as parameters. An increase in percentage of α₂ and γ-globulin fractions, gain in body weight and alertness in physical behavior was observed after the treatment (Gaur et al., 1968).
The alcoholic extract of the dried leaves (200mg/kg) was reported to increase wound healing concentration rate, skin breathing strength, granuloma breaking
strength, hydroxyproline content and dried granuloma weight significantly (Shirwaikar et al., 2002).

3.2.2 Clinical reviews:

The wine extract of roots of *G. arborea*, was reported to increase milk secretions when given orally to adult female (Petelot, 1954). Dried roots in the form of decoction were found to possess anthelmintic, laxative and appetite stimulant activity, however decoction of fresh roots also exhibited laxative activity and found to be used as a demulcent in the treatment of gonorrhea and as a bitter tonic when administered orally (Shah, 1985).

The hot water extract of plant was found effective in the treatment of cough and gonorrhea when given orally. It was also reported to be used orally in the treatment of tuberculosis in human adult (Joshi et al., 1977).

Hot aqueous extract of dried flowers were reported to be used as an astringent and in the treatment of leprosy when applied externally. The fresh flowers of the plant were reported to be used as a food material in China (Deka et al., 1983, Pei, 1985). Further investigation revealed that leaves were also used as a food material in tribes of Madurai district (Ignacimuthu et al., 2006). The decoction of dried fruit was found to be effective as hair growth promoter and as a general tonic when administered orally in human adults (Deka et al., 1983).

Ethnobotany survey on dried inner bark powder showed that, when applied externally, it was used in the treatment of scabies (Alam, 1992). In one of the reports for the survey of antisnake venom botanicals, this plant was mentioned to be used in snakebite and scorpion sting (Selvanyahgam et al., 1994).
3.2.3 Phytochemical reviews:

Lignans:

*G. arborea* Roxb. has been reported for the presence of number of novel lignans from heartwood. Two new, tetrahydrofuranoid lignan, arboreol and gmelinol were isolated from the heartwood (Birch et al., 1954, Birch et al., 1967) Further arboreol was confirmed as 1-hydroxy-2-methoxy, 2,6 bis (3,4 methylenedioxy phenyl)-3-7 dioxobicyclo (3,3,0) octane, by a combination of spectral and degradative evidences and mass spectral fragmentation pattern of arboreol was established (Pelter, 1967). Oxidation, hydrogenolysis, action of acid and action of NaIO$_4$ on arboreol were also studied. This was the first instance of a naturally occurring tetrahydrofuranoid lignan substituted at the benzyl carbon (Govindachari et al., 1972). Further phenolic rearrangement from arboreol and isoarboreol yielded gmelanone (Birch et al., 1967). Studies of acid catalyzed rearrangements of arboreol revealed that this reaction confirmed the biosynthesis and absolute configuration of gmelanone. Gmelanone was found unstable and believed to undergo a ring opening reaction upon acid exposure. This was the only instance of naturally occurring lignan with a rearranged carbon skeleton (Row and Ventkateshwarlu, 1980).

Isolation of two known furofuran lignans, paulownin acetate (0.007%) and epieudesmin (0.001%) from the heartwood was reported (Freudenberg and Sidhu, 1960, 1961; Takahashi et al., 1963).

Few lignans were quantified and their structures were determined, from heartwood of *G. arborea*. This included arboreol (0.00560%), arboreol 2-O-ethyl, arboreol 2-O-methyl (0.0035%), isoarboreol (0.004%), gmelanone, gmelinol and paulownin found along with β-sitosterol (Anjaneyulu et al., 1975).

Gummadiol, a structural isomer of arboreols, was isolated along with paulownin and other gummadiol derivative. Gummadiol was characterized as 1,4 dihydroxy -2-6 dipiperonyl 1-3-7 dioxabicyclo-(3, 3, 0)-octane, and reported to be a first member of new series of hydroxy lignans (Anjaneyulu et al., 1975a).

6"-bromo-isoarboreol, the first bromine containing lignan was characterized as 1,2a-dihydroxy piperonyl 6-e-(6"-bromopiperonyl)-3,7 dioxabicyclo piperonyl
Review of Literature

-e(6'-bromopiperonyl)-3-7 dioxabicyclo (3,3,0)-octane. This was a rare example of halogen containing product isolated and derived from a higher plant (Anjaneyulu et al., 1975b).

Novel hydroxy lignans were isolated from heartwood of *G. arborea* and were characterized as sesamin 4,8 dihydroxy (0.005%), sesamin 4 -hydroxy (0.005%), Furan, tetrahydro 2-piperonyl-3-hydroxy-methyl-4-(alpha-hydroxy-3-4-methylene dioxy (0.005%), Gummadiol(0.61%), Gummadiol 4-epi; 4-o-β-D-glucose (0.025%) (Anjaneyulu et al., 1977).

Two lignans named 2, 3, 4-trisubstituted tetrahydrofuranoid lignans, arborone (0.00025%) and 7-oxo-dihydrogmelolin (0.0002%) were isolated from the heartwood. Two new furofuran lignans, paulownin acetate (0.007%) and epieudesmin (0.001%), were isolated along with methyl trans-p-methoxycinnamate and trans-p-hydroxycinnamic acid (0.0015%) (Satyanarayana et al., 1986).

Twelve acylated iridoid glycosides named gmelinosides A-L were isolated and characterized from leaves along with 6-O-α-L-rhamnopyranosylcatalpol, 6-O-(3’’-O-trans-reruloys)-α-L-rhamnopyranosyl-catalpol, 6-O-(2”’-O-acetyl-3”,4”'-O-di-trans-cinnamoyl)-α-L-rhamno-pyranosylcatalpol (Hosny and Rosazzza, 1999).

**Flavonoids:**

The method for isolation of luteolin from ether extract of leaves of *G. arborea* (m.p. 322-25°C) was reported (Rao et al., 1967). Extended chemical examination of leaves revealed that the alcoholic extract gave positive test for flavones and yielded three flavonoids where luteolin (m.p. 322-25°C) was the major component and apigenin (m.p. 348-50°) and quercetin (m.p. 318-22°C) were minor components. Further hentriacontanol (m.p. 87°C) was found to be present in petrol ether extract and β-sitosterol (m.p. 136-38°C) in benzene extract of leaves (Rao et al., 1970).

A flavonol of rare occurrence, quercetagetin was reported in leaves along with other flavonol glycosides, kaempferol 3- rutinoside, nicotiflorin, and flavone isorhoifolin, luteolin, apigenin, luteolin 7-β glucuronide, apigenin 7-o-β-glucuronide and apigenin -7- rutinoside (Nair and Subramanian, 1975).
**Terpenoids:**

*G. arborea* yielded a sesquiterpene along with ceryl alcohol and β-sitosterol from neutral fraction of ether soluble petroleum extract of roots. Hentriacontanol was isolated from acidic fraction of light petroleum ether extracts, while ether insoluble portion gave n-octacosanol and gmelinol was found to be present in aqueous extract of roots (Joshi et al., 1977).

A unique cadinane type furanosesquiterpenoid, gmelofuran (m.p. 122-23°C) from petrol ether extract of roots, was isolated and characterized as 5-isopropyl-7 methyl-5,5a,6,7,-tetra-hydro-3H, 8H-naphtho (1,8-bc) furan-3, 8(4H)-dione (Joshi et al., 1978).

Nonvolatile dichloromethane extractives of wood yielded C18-C30 wax alcohols, C28 and C30 (14.7%) predominating along with β-sitosterol, stigmasterol, stigmastanol, campesterol, α2-sitosterol and bêutulinol was found to be present in non saponifiable fraction. Saponifiable fraction contained oleic acid, linoleic acid and relatively high amount of saturated fatty acids (Ukkonen, 1982).

**Other phenylpropanoid:**

A new long chain ester, cluytyl ferulate (0.04 %, m.p. 80-82°C) from the heartwood of *G. arborea* along with cluytyl alcohol were isolated and the structure of ester was established by spectral studies and synthesis from ferulic acid and cluytyl alcohol (Govindachari et al., 1971).

A rare example of an apiose containing coumarin glycoside from roots of *G. arborea*, was reported and named as apiosylskimmin (m.p. 141-42°C) (Satyanarayana et al., 1985).

Phenylpropanoids like calceolarioside A, verbascoside (0.241%) and isoverbascoside were found to be present in leaves during the screening for caffeic acid heteroside esters forms in Verbenaceae (Taoubi et al., 1992).

**Miscellaneous:**

Alkaloid screening of various plants of family Verbenaceae revealed presence of alkaloids in fruits of *G. arborea* (Smolenski et al., 1975).

During the survey on screening for the presence of pyrrolizidine alkaloids and hepatotoxic properties in some medicinal plants, results indicated that pyrrolizidine alkaloids were found to be absent from this plant (Arseculeratne et al., 1981). An isoxazole alkaloid, premnazole (0.05%) with anti-
inflammatory activity was isolated from leaves of *G. arborea*. The activity was found comparable with phenylbutazone in reducing cotton-pellete granuloma formation in rats, probably by regulating activity of ACTH (Barik et al., 1992).

The chemical composition of Nigerian wood species of this plant has been reported for the presence of various carbohydrates like arabinose, galactose, d-glucose, d-mannose, rhamnose and xylose (Sosanwo and Lindberg, 1975). Further, fatty acid composition of seed oil in Nigerian species showed presence of arachidic acid, lauric acid, linoleic acid, linolenic acid, myristic acid, oleic acid, palmitic acid and stearic acid (Adeyeye, 1991).

### 3.2.4 Pharmacognostic reviews:

In a light microscope study of the secondary phloem in *G. arborea*, many sieve elements were found to possess bar-shaped cytoplasmic inclusions of proteinaceous nature. It was suggested that these inclusions represent a type of crystalline P-protein not reported in the family Verbenaceae ever before (Vishvakarma and Deshpande, 1990).

In *G. arborea* Roxb, abundance of acicular crystals were found to be present in fusiform cells of cambium and a correlation between the presence of crystals and cambium activity in *G. arborea* was reported. This was observed with seasonal variation, decreasing in abundance during the meristematic activity. In dormant cambium, cell divisions were found virtually nonexistent; there was no cell expansion observed and radial rows of fusiform cells became more compact (Deshpande and Vishwakarma, 1992).
3.3 Pathophysiology of liver disorders

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism, weighing roughly 1.2-1.6 kg and performs many of the functions necessary for staying healthy. This organ is the largest gland in the human body and comprising up to 2% of the body weight of an adult human. A liver lobule is the basic repeating functional unit of the liver, which contains liver cells, blood vessels, bile ducts, and macrophages. Hepatocytes radiate out from the central vein and as blood passes through, they are able to monitor, add and remove substances from it. The blood then leaves the liver via the hepatic vein, returns to the heart, and is ready to be pumped to rest of the body (Edoardo et al., 2005).

The most important liver functions are:

**Excretes body wastes, hormones, drugs and other foreign substances:** which enters either through production by metabolism within the body or from the outside in the form of drugs or other foreign compounds.

**Synthesizes plasma proteins, including those necessary for blood clotting:** Most of the 12 clotting factors are plasma proteins produced by the liver. The liver produces coagulation factors I (fibrinogen), II (prothrombin), V, VII, IX, X and XI, as well as protein C, protein S and antithrombin.

**Produces immune factors, removes bacteria and helps the body to fight infection:** The phagocytes in the liver produce acute-phase proteins in response to microbes. These proteins are associated with the inflammation process, tissue repair and immune cell activities.

**Produces bile to aid in digestion:** Bile is continuously secreted by liver and it aids the digestion of fats and promotes absorption from the intestinal lumen.

**Excretion of bilirubin:** Bilirubin is one of the few waste products excreted in bile. Jaundice results when bilirubin cannot be removed from the blood quickly enough which may be due to gallstones, liver disease, or the excessive breakdown of red blood cells.

**Stores certain vitamins, minerals and sugars:** The liver stores enough glucose in the form of glycogen and also stores fats, iron, copper and many vitamins including vitamins A, D, K and B₁₂.
Processing on nutrients absorbed from digestive tract:

**Carbohydrate metabolism:** The liver performs several roles in carbohydrate metabolism and also uses some of the byproducts of carbohydrate metabolism to form various chemical compounds necessary for other physiologic functions. It also regulates breakdown of insulin and other hormones.

**Fat metabolism:** The liver is a major organ in the formation of cholesterol, processing of dietary fat and conversion of stored fat into products more readily used for energy.

**Protein metabolism:** The liver functions to remove the nitrogen group from amino acids, forms urea from the excess ammonia produced in that process, form various proteins (especially albumin) and also form some amino acids for protein production (Porth, 1998).

Liver is relatively sturdy organ that is less liable to diseases but once it get diseased it is difficult to treat. It has remarkable power of regeneration and self-repairs after being injured or diseased. An imbalance between liver cell death and regeneration leads to hepatic injury and subsequently to its failure.

A large number of disorders can affect the liver and interfere with the blood supply, hepatic and kupffer cells and bile ducts. The term "liver disease" applies to many disorders that cause the liver to function improperly or cease functioning. Liver has an inherent capacity to convert a wide variety of foreign substances, drug and poisons to more readily excreted derivatives, unfortunately in some instances this detoxification system fails. A number of otherwise useful drugs when taken in either therapeutic or somewhat higher doses sometimes produce massive liver injury (Araya et al., 1987). Toxic drug molecule or its metabolites can produce direct cellular injury to the liver or otherwise interfere with its functions and leading to incurable hepatic disorders and disease (Date et al., 1997). Some autoimmune diseases are also known to produce hepatic disorders (Vander et al., 1998).

About 20,000 deaths occur every year due to liver diseases. Hepatocellular carcinoma is one of the ten most common tumours in the world with over 2,50,000 new cases each year. Although viruses are the main cause of liver diseases, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon.
Various antibiotics, chemotherapeutic agents, peroxidized oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbon etc are some of the known hepatotoxic agents (Subramanium and Pushpangandan, 1999).

Oxidative stress is another major cause of liver disease. Irrespective of the type of causative agent, inflammatory changes occur in the liver (hepatitis) and depending upon the duration and severity of inflammation, cellular changes occur in the liver leading to the liver damage (necrosis) with infiltration of fats (fatty liver) or fibrosis (liver fibrosis). All these alterations may finally lead to cirrhosis of liver. Clinical manifestations of various pathological alterations of liver include accumulation of bilirubin in the blood (jaundice), enlargement of liver, altered biochemical mechanisms like SGOT, SGPT, alkaline phosphatase, albumin etc. All these finally lead to hepatic encephalopathy and cirrhosis of liver and finally to hepatic coma and death. Liver disease is often reflected by biochemical abnormalities of different hepatic systems (Fig:2).

Although tests that measure the level of serum liver enzymes are commonly referred to as liver function tests, they usually reflect hepatocytes integrity or cholestasis rather than liver function (Edoardo et al., 2005).

<table>
<thead>
<tr>
<th>System or function</th>
<th>Marker</th>
<th>Site or significance</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocyte integrity</td>
<td>Aspartate aminotransferase</td>
<td>Liver, heart, skeletal muscle, kidney, brain, red blood cell</td>
<td>Catabolizes amino acids, permitting them to enter the citric acid cycle.</td>
</tr>
<tr>
<td></td>
<td>Alanine aminotransferase</td>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Cholestasis</td>
<td>Alkaline phosphatase</td>
<td>Bone, intestine, liver, placenta</td>
<td>Canicular enzyme that plays a role in bile production</td>
</tr>
<tr>
<td></td>
<td>γ-Glutamyl-transpeptidase</td>
<td>Correlated levels with alkaline phosphatase indicate hepatobiliary origin</td>
<td>Catalyzes transfer of γ-glutamyl group from peptides to other amino acids.</td>
</tr>
<tr>
<td></td>
<td>Bilirubin</td>
<td>Elevations may indicate hepatic or extrahepatic disorder</td>
<td>Breakdown product of hemolysis taken up by liver cells and conjugated to water-soluble product excreted in bile</td>
</tr>
<tr>
<td>Liver function mass</td>
<td>Serum albumin</td>
<td>Diet or liver</td>
<td>Liver synthesizes albumin</td>
</tr>
<tr>
<td></td>
<td>Prothrombin time</td>
<td>Liver synthesizes vitamin K-dependent clotting factors</td>
<td>Bile salts are synthesized in the liver and necessary for vitamin K absorption</td>
</tr>
</tbody>
</table>

Fig: 2 Key biochemical markers in hepatic system and functions
The most common alteration in enzyme levels can be divided into two major subgroups: hepatocellular predominant and cholestatic predominant. Although certain liver diseases may display a mixed biochemical picture usually elevated AST and ALT levels with mild abnormalities of alkaline phosphatase and bilirubin. AST and ALT are enzymes that catalyze the transfer of α-amino groups from aspartate and alanine to the α-keto group of ketoglutaric acid to generate oxaloacetic and pyruvic acids respectively. Both enzymes require pyridoxal-5'-phosphate (vitamin B₆) in order to carry out this reaction, although the effect of pyridoxal-5'-phosphate deficiency is greater on ALT activity than on that of AST (Vanderlinde, 1986; Dufour et al., 2000).

Both aminotransferases are highly concentrated in the liver. AST is also diffusely represented in the heart, skeletal muscle, kidneys, brain and red blood cells and ALT has low concentrations in skeletal muscle and kidney; (Wroblewski, 1958), an increase in ALT serum levels is, therefore, more specific for liver damage. In the liver, ALT is localized solely in the cellular cytoplasm, whereas AST is both cytosolic (20% of total activity) and mitochondrial (80% of total activity) (Rej, 1989).

Presentation of liver injury with a prevalent cholestatic pattern is less frequently encountered in clinical practice than the pattern of hepatocellular damage. ALP and bilirubin levels are routinely assessed and the level of GGT is often measured as an additional aid towards diagnosis in particular situations because of its high sensitivity but low specificity.

Alkaline phosphatase (ALP) is an enzyme that transports metabolites across cell membranes. Liver and bone diseases are the most common causes of pathological elevation of ALP levels (Fishman, 1990). Hepatic ALP is present on the surface of bile duct epithelia. Cholestasis enhances the synthesis and release of ALP and accumulating bile salts increase its release from the cell surface (Schlaeger et al., 1982; Moss, 1997).

GGT is an enzyme that is present in hepatocytes, biliary epithelial cells, renal tubules, pancreas and intestine. The mechanisms of alteration are similar to those described for alkaline phosphatase. GGT is a microsomal enzyme and its activity can be induced by several drugs such as anticonvulsants and oral contraceptives (Rosalki et al., 1971).
Bilirubin is the product of hemoglobin catabolism within the reticuloendothelial system. Heme breakdown determines the formation of unconjugated bilirubin, which is then transported to the liver. In the liver, UDP-glucuronyltransferase conjugates the water-insoluble unconjugated bilirubin to glucuronic acid and conjugated bilirubin is then excreted into the bile (Fevery and Blanckaert, 1986; Berk and Noyer, 1994). Unconjugated bilirubin may increase because of augmented bilirubin production or decreased hepatic uptake or decreased conjugation or both. In adults, the most common conditions associated with unconjugated hyperbilirubinemia are haemolysis and Gilbert’s syndrome (Fevery and Blanckaert, 1986). In healthy people, conjugated bilirubin is virtually absent from serum mainly because of the rapid process of bile secretion. Levels increase when the liver has lost at least half of its excretory capacity. Therefore, the presence of increased conjugated bilirubin is usually a sign of liver disease. Conjugated hyperbilirubinemia (usually<34μmol/L) and concomitant, markedly elevated aminotransferases levels may suggest acute viral hepatitis or toxic or ischemic liver injury (Green and Flamm, 2002).

The ineffectiveness of modern therapeutic agents in completely curing hepatic disorders has been lamented. The remedies available in modern medicine provide symptomatic relief, without any significant changes to the etiological causes of the disease process. Moreover, as many therapeutic agents are known to cause conditions such as liver cirrhosis and fulminant hepatic failure, the development or identification of new molecules effective in treating or preventing hepatic damage remains a challenge in the field of drug development.

The commonly occurring liver diseases include cirrhosis, hepatitis, liver abscesses and pediatric liver diseases. Other serious diseases of the liver include fatty liver, hepatic coma and liver cancer. Causes of liver damage include drug overdose, metabolic and autoimmune disorders, many chemical drugs, alcohol abuse and trauma. Liver failure can progress extremely rapidly as in fulminant hepatic failure (FHF), or slowly as with chronic liver diseases.
3.3.1 Hepatitis:

Hepatitis is an inflammation of the liver, caused mainly by various viruses but also by some poisons, autoimmunity or hereditary conditions. Viral hepatitis (A, B, C, D, E; and G) is a contagious infection of liver usually caused by one of three different organisms. Hepatitis A, formerly known as infectious hepatitis, can be contracted by consuming contaminated water or food, most notably shellfish. The virus is eliminated in stool and though seldom serious it can cause severe liver failure and death. It does not cause chronic hepatitis and does not lead to cirrhosis or other long-term liver problems. Hepatitis B, formerly known as serum hepatitis, is found in blood and other body fluids such as urine, tears, semen, breast milk and vaginal secretions. It is usually transmitted in blood, via transfusion or through injectable-drug use. Type C hepatitis virus is the cause of a disease known as "non-A, non-B hepatitis" which is also contracted through contact with infected person. Viral hepatitis may produce no symptoms at all. Symptoms of viral hepatitis mimic flu. Hepatitis D virus occurs in individual with hepatitis B. The delta virus depends on the hepatitis B virus for its replication because the coat of delta virus consists of HBsAg molecules that are on the surface of the HBV virus. Hepatitis E is the most common in developing countries and is transmitted by the fecal-oral route usually by contaminated water. It is more prevalent among adults and has the highest mortality in pregnant women. Clinically it resembles with HAV.

Hepatitis G virus is a newly identified virus in people who had a blood transfusion developed ‘post transfusion hepatitis’ that could not be identified as any known virus. HGV is an RNA virus similar to, but distinct from, the HCV. Infection with the HGV virus can lead to persistent infection in 15 – 30% of adults and there is no vaccination available for hepatitis G. Leading causes of chronic liver disease are viral hepatitis and alcohol abuse. Alcoholic liver disease involves an acute or chronic inflammation of the liver induced by alcohol abuse. Changes start within the liver as inflammation (hepatitis) and progress to fatty liver and cirrhosis.

Alcoholic hepatitis is an inflammatory response of the liver to chronic alcohol ingestion. The intensity of the response varies from anicteric asymptomatic individuals who have a subclinical alcoholic hepatitis to individuals with
jaundice, ascites and hepatic encephalopathy. Alcoholic hepatitis can lead to alcoholic cirrhosis, but it is not always a precursor of cirrhosis. Fatty liver is reversible with cessation of alcohol consumption. However, any necrosis or scarring associated with the fatty liver is permanent. Alcoholic hepatitis can produce jaundice of the hepatocellular type. Cirrhosis is the final phase of alcoholic liver disease. Serious complications are associated with advanced disease such as alcoholic encephalopathy (damage to brain tissue) and portal hypertension (high blood pressure within the liver).

Drug Induced Hepatitis reflects by two adverse reactions of the liver to drugs:

- Intrahepatic (medical) cholestasis is caused by impaired formation of canalicular bile by slowing of active transport by hepatocytes. Hepatocellular damage ranging from mild inflammation to fulminant massive necrosis. Chronic hepatitis is most often associated with viral agents, chemicals and alcohol. There are two kinds of chronic hepatitis:
  - Chronic active liver disease (CALD) is aggressive and often leads to cirrhosis (30-50%) and has high mortality rates.
  - Chronic persistent hepatitis is a benign disease that does not progress to cirrhosis, nor is there any increased mortality.

3.3.2 Cirrhosis:

Cirrhosis is a diffuse fibrosis that replaces normal parenchyma and ultimately changes the normal lobular pattern to nodules of liver cells surrounded by scar tissue and hardens the liver, diminishes blood flow and causes even more cells to die. The death of the liver cells can be caused by viral hepatitis, alcoholism or contact with other liver-toxic chemicals. Fibrosis ranges in density from delicate almost wispy fibers to massive scarring. Nodule formation is always present when fibrous tissue surrounds normal parenchymal cells. Cirrhosis is a progressive disorder that leads to complications such as portal hypertension, ascites (abnormal volume of serous fluid within the peritoneal cavity), gastrointestinal disturbances, jaundice, enlargement of liver and spleen, maceration and accumulation of fluid in the abdomen and other tissues and ultimately causes liver failure. It has been reported that 60-70% of the cirrhosis is associated with alcohol abuse (Laennec’s Cirrhosis). Alcohol
abuse, hepatitis, chemical poisoning, excess of iron or copper, other viruses and blockages of the bile duct can cause the disease.

The exact prevalence of cirrhosis is unknown, but it has been estimated, through autopsies, to be between 5 and 10 percent. Incidence of cirrhosis varies by country and region and reflects relative contributions from different risk factors. In countries where alcohol consumption is common, alcoholic cirrhosis is the major contributor to the overall prevalence of cirrhosis. In countries with low alcohol consumption, hepatotropic viruses (hepatitis B and C) are the major contributors (Owais et al., 2006).

Alcohol is the most common cause and the amount necessary to cause cirrhosis differs, based on gender and nutritional status. The relative risk of alcoholic cirrhosis increases with greater amounts of alcohol consumption. Alcoholic hepatitis is a precursor of cirrhosis characterized by inflammation, degeneration and necrosis of hepatocytes and infiltration of polymorphonuclear leukocytes and lymphocyte. The injured hepatocytes contain Mallory bodies (hyaline endoplasmic reticulum). The presence of Mallory bodies indicates the onset of fibrosis. The inflammation and necrosis caused by alcoholic hepatitis stimulate the fibrosis characteristic of cirrhotic stage of disease (Zetterman, 1996).

Hepatotropic viruses represent the second major category of the causes of cirrhosis. Hepatotropic viruses account for most orthotropic liver transplantations in the United States. Hepatitis C infection results in chronic hepatitis in 85 percent of infected individuals and in cirrhosis in 20 percent. The mean time progression to hepatic cirrhosis following viral infection is twenty years.

Biliary cirrhosis differ form alcoholic cirrhosis in a way that the damage and inflammation leading to cirrhosis begin in bile canaliculi and bile ducts, rather than in the hepatocytes. Primary biliary cirrhosis is the autoimmune disease of small bile ducts. Both Primary and secondary biliary cirrhosis involve bile duct pathology, they differ with respect to cause, risk factors and mechanisms of obstruction and inflammation. Primary biliary cirrhosis causes inflammation and destruction of intrahepatic bile ducts. It is characterized by itchy skin and fatigue, jaundice, cholesterol deposits on the skin, fluid retention and dry eyes or mouth and cirrhosis, symptoms of portal
hypertension and encephalopathy and ultimately liver failure develop. Secondary biliary cirrhosis develops when there is prolonged partial or complete obstruction of the common bile duct or its branches. The obstruction increases pressure in bile ducts and results in the accumulation of bile that leads to edema and fibrosis and finely to cirrhosis (Owais et al., 2006).

### 3.3.3 Jaundice (Icterus)
Jaundice is a yellow coloring of the skin and eyes that occurs with disorders of bilirubin metabolism and/or excretion. This is due to the presence of excessive amounts of the yellow-brown pigment bilirubin in body fluids and also known as hyperbilirubinemia. Jaundice is typically not present until the total bilirubin concentration exceeds about 3 mg/dl.

**Extrahepatic obstructive jaundice** develops if the common bile duct is occluded by a gallstone or tumour and bilirubin cannot flow into the duodenum. Therefore it accumulates into the liver and enters the bloodstream, causing hyperbilirubinemia.

**Intrahepatic obstructive jaundice** involves disturbance in hepatocytes function and obstruction of the bile canaliculi. The uptake, conjugation and excretion of bilirubin are affected because of elevated levels of both conjugated and unconjugated bilirubin. Obstruction of bile canaliculi diminishes flow of conjugated bilirubin into the common bile duct (Raiford, 1995).

Excessive haemolysis of red blood cells can cause hemolytic jaundice. An increased amount of unconjugated bilirubin is formed through metabolism of the heme component of destroyed red blood cells. The extra amount of unconjugated bilirubin exceeds the conjugation ability of the liver, causing blood levels of unconjugated bilirubin to rise and if it exceeds 5 mg/dl, both hemolytic and liver disorders are indicated (Nenberger, 1989).

Liver abscesses are caused by bacteria such as *Escherichia coli*, *Staphylococcus*, or *Entamoeba histolytica* and result in destruction of liver tissue) leaving a cavity that fills with other infectious organisms, white blood cells and liquefied liver cells. Symptoms include pain, fever, jaundice and anemia.

There are about 100 pediatric liver diseases most of which are genetic
including biliary atresia (inadequate bile duct), alpha-1 antitrypsin, alagille syndrome, progressive familial intrahepatic cholestasis and often fatal, chronic active hepatitis. Wilson’s disease is characterized by abnormally large build-up of copper in the liver; and Reye’s syndrome, in which fat accumulates in the liver and the patient lapses into coma. Some other liver disorders include, Hemochromatosis, (hereditary accumulation of iron in the body), liver cancer, primary sclerosing cholangitis (inflammatory disease of the bile duct, autoimmune in nature). Budd-Chiari syndrome is obstruction of the hepatic vein and Gilbert’s syndrome is a genetic disorder of bilirubin metabolism. In glycogen storage disease type II, excess glycogen causes progressive muscle weakness (myopathy) throughout the body and affects various body tissues, particularly in the heart, liver and nervous system (Owais et al., 2006).

Hepatorenal syndrome consists of advanced liver disease and functional renal failure with oliguria, sodium and water retention, hypotension and vasodilation. Renal disorders associated with liver disease can have numerous causes, but hepatorenal syndrome is usually associated with alcoholic cirrhosis and fulminant hepatitis.

Hepatic encephalopathy (protosystemic encephalopathy is a complex neurologic syndrome characterized by impaired cerebral function, flapping tremor and electroencephalogram changes. The syndrome may develop rapidly during acute fulminant hepatitis or slowly during the course of chronic liver disease. Risk factors in the presence of advanced liver disease include gastrointestinal bleeding, increased dietary protein, electrolyte imbalance and hypoxia. Liver dysfunction and collateral vessels that shunt blood around the liver to the systemic circulation both permit toxins absorbed from the gastrointestinal tract to circulate freely to the brain. The most hazardous substances are end products of intestinal protein digestion, particularly ammonia. But only this do not account for all symptoms associated with hepatic encephalopathy. The accumulation of short chain fatty acids, serotonin, tryptophan and false neurotransmitters probably contributes to neural derangement (Scafer and Jones, 1990).
3.4 Free radicals and reactive oxygen species (ROS):

A free radical is an atom, molecule, or compound that is highly reactive as it attempts to pair up with other molecules, atoms, or even individual electrons to create a stable compound. Any element that has an unpaired electron in its outermost shell is considered to possess a "free radical" (Harman, 1956; Defeng et al, 2003).

Reactions involving free radicals

The four primary types of chemical reactions that free radicals undergo are:

- **Hydrogen abstraction**, in which a radical interacts with another molecule that has a free hydrogen atom and becomes stable, whereas the hydrogen donor is converted to a free radical.
- **Addition**, in which radical binds to another, originally stable molecule, converting the combined molecule into a radical.
- **Termination**, in which two radicals react with each other to form a stable compound.
- **Disproportionation**, in which two identical radicals react with each other, with one of the radical donating electron to the other so that two different molecules are formed, each of which is stable (Defeng et al., 2003).

Free radicals are usually named after the atom, which harbors an additional electron. Accordingly, the radicals are classified as oxygen/nitrogen/sulphur/carbon centered radicals. Sometimes, the radicals containing oxygen and their subsequent radical products are collectively called as reactive oxygen species. In similar way some are termed as reactive nitrogen species (RNS) and carbon containing aryl radical (R*) (Mason, 1980).

The most important free radicals in biological systems are derivatives of oxygen, derived by its reduction. During this process, different oxygen radicals are successively formed as intermediate products, including superoxide (O$_2^-$), peroxide (O$_2^-$), which normally exists in cells as hydrogen peroxide (H$_2$O$_2$) and the hydroxyl radical (OH$^*$). Superoxide, peroxide and the hydroxyl radical are considered the primary ROS and have sparked major research on the role of free radicals in biology and medicine (Defeng et al., 2003). However, because they are unstable and rapidly react with additional
electrons and protons, most of these ROS are converted to water before they can damage cells. It has been estimated that only about 2 to 3 percent of the O\textsubscript{2} consumed by the respiratory chain is converted to ROS. Further not all free radicals are ROS and not all ROS are free radicals. For example, the free radicals superoxide and hydroxyl radical are ROS, but the ROS hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) is not a free radical species. Non radical reactive species include singlet oxygen (\textsuperscript{1}O\textsubscript{2}), hypochlorous acid (HOCl) and ozone (O\textsubscript{3}) (Harman, 1956; Chance et al., 1979).

### 3.4.1 Sources and types of free radicals:

Examples of different sources for generating free radicals are shown in Table:1 (Lillian, 1995; Mark, 1998).

<table>
<thead>
<tr>
<th>Sources of free radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Some sources of free radicals</strong></td>
</tr>
<tr>
<td><strong>Internally generated sources</strong></td>
</tr>
<tr>
<td>Mitochondria</td>
</tr>
<tr>
<td>Phagocytes</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
</tr>
<tr>
<td>Reactions involving iron and other transition metals</td>
</tr>
<tr>
<td>Arachidonate pathways</td>
</tr>
<tr>
<td>Peroxisomes</td>
</tr>
<tr>
<td>Exercise</td>
</tr>
<tr>
<td>Inflammation</td>
</tr>
<tr>
<td>Ischemia/reperfusion</td>
</tr>
<tr>
<td><strong>External sources</strong></td>
</tr>
<tr>
<td>Cigarette smoke</td>
</tr>
<tr>
<td>Environmental pollutants</td>
</tr>
<tr>
<td>Radiation</td>
</tr>
<tr>
<td>Ultraviolet light</td>
</tr>
<tr>
<td>Certain drugs, pesticides, anesthetics and industrial Solvents</td>
</tr>
<tr>
<td>Ozone</td>
</tr>
</tbody>
</table>

Superoxide radicals are beneficial because they destroy virus, bacteria and cancer cells. It also increases the activity of epinephrine and norepinephrine, necessary for fight-or-flight response, in large amounts; however, it can damage lipids, proteins and DNA.

Hydroxyl radicals: Hydroxyl radicals are produced by cells up on direct exposure to gamma rays or X-rays through sunlight. They are the most deadly,
highly reactive and powerful, destroying everything in its path by altering DNA and contributing to plaque formation in blood vessels.

*Hydrogen peroxide* is a by-product of the degradation of fats during energy production. Because fat cells divide rapidly there is a greater risk of mutation in fatty tissue (Jampol, 2001).

*Nitric oxide* is produced in the body through enzymatic reactions. Although it is beneficial to the body at low levels because it regulates both blood flow in the vessels and blood pressure, but in excess it can cause damage (Merrily, 2003).

**Organic radicals in the metabolism of xenobiotics:**

The organic radicals likely to be produced in the metabolism of xenobiotics are C-, N-, O-, or S-centered (Henderson et al., 1989). The C-centered radicals are produced almost exclusively by cytochrome P<sub>450</sub> dependent mixed function oxidases (Goeptar et al., 1995; Lieber 1997), the S-centered radicals are products of both cytochrome P<sub>450</sub> enzymes and peroxidases, mainly prostaglandin H synthase; the formation of O-centered radicals is catalyzed mainly by peroxidases (Slater et al., 1995), but the production of nitroxyl and semiquinone radicals can also be catalyzed by cytochrome P<sub>450</sub> enzymes and flavoproteins (Breitkreutz et al., 2000); the metabolism of amines, amides and hydrazines to produce N-centered radicals is a property of peroxidases. The one electron reduction of azo compounds to form the azo anion radicals involves a flavoprotein and the formation of the nitro anion radical during the metabolism of nitroaromatics is catalyzed by both peroxidases and flavoproteins (Kirkman et al., 1987).

**Coenzyme-derived radicals:**

The single electron oxidation of NAD(P)+ may produce NAD(P)⁺ radical (Shaaltiel and Gressel, 1986) the free form of NADH is rather resistant to such a one-electron transfer, but when it is bound to an enzyme, as in lactate-NAD⁺ oxidoreductase, it becomes particularly sensitive and can be dimerized (Hofstra and Uetrecht, 1993). FAD is also a photosensitizer that may participate in energy transfer so as to produce the singlet oxygen (Herttog et al., 1993).
Glutathionyl radicals:

Glutathione (GSH) is an endogenous molecule which can be oxidized to give the thiyl radical (GS), when it reacts with the superoxide anion ($\text{O}_2^-$), hydroxyl radical ($\text{OH}^*$) or molecular oxygen in the presence of a transition metal ion (Richman and Meister, 1975). The thiyl radical (GS) may further react with $\text{O}_2$ to form the glutathione peroxyl radical (GSO$_2^*$) and also dimerized to give oxidized form of glutathione (GSSG).

Uric acid derived C-centered radicals:

Uric acid serves as a hydroxyl radical scavenger, a reaction that produces a C-centered radical to which $\text{O}_2$ can add to form a peroxyl radical (Amens et al., 1993).

3.4.2 ROS and oxidative stress

Reactive oxygen species (ROS) is a collective term, which include not only oxygen radicals but also some derivatives of oxygen that do not contain unpaired electron such as singlet oxygen ($^1\text{O}_2$), hydrogen peroxide ($\text{H}_2\text{O}_2$) (Simonian and Coyle, 1996). Thiyl (RS, a sulphur-centered radical), trichloromethyl (CCl$_3^*$, a carbon-centered radical) and nitric oxide (NO), all are capable to cause cellular damage (Halliwell and Gutteridge, 1990; Sen, 1995; Parihar et al., 1997).

The etiological role of free radicals, in carbon tetrachloride induced hepatotoxicity has been well demonstrated. This has been further investigated for the involvement of free radicals in characteristic tissue degeneration in several diseases. This pioneer work encouraged several workers to define the role of free radicals in the pathophysiology of many degenerative diseases. The simultaneous biochemical research furnished voluminous information about the types, genesis, fate and the biochemical physiological significance of radicals, which helped several workers to successfully delineate pathological conditions with free radicals (Slater, 1966).

Free radicals are molecular sharks that damage molecules in cell membranes, mitochondria, DNA and this appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, brain and liver dysfunction (Sies, 1992; de Groot, 1994; Nakazawa et al., 1996, Toykuni 1999).
This involvement is not at all surprising as free radical chemistry is an important aspect of phagocytosis, inflammation and apoptosis. Apoptosis is the body's way of controlling cell death and it involves free radicals and redox signaling. Redox factors play an even greater part in other forms of cell death such as necrosis or autoschizis (Harman, 1956). Hyper physiological burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. This imbalance leads to oxidative stress that is being suggested as the root cause of aging and various human diseases like atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's disease and Parkinsonism. Therefore, in modern western medicine, the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system (Tiwari, 2004).

Most of the systems for the production of ROS produce superoxide or hydrogen peroxide radicals, which interacts with each other to produce the hydroxyl radical (OH\(^*\)). Under normal physiological conditions, direct interaction between these two radicals is not likely to play a significant role in generating hydroxyl radicals. However, in the presence of certain metals, particularly free iron or copper ions, a sequence of two reaction steps can occur that results in hydroxyl radical generation. In the first step, hydrogen peroxide produces hydroxyl radical by removing an electron from the participating metal ion and in second step, involving the superoxide radical (O\(_2\)\(^*\)), the original metal ions are regenerated so that they are again available for reaction with the hydrogen peroxide. This accounts for most of the hydroxyl radical production in biological systems and explains, at least in part, why metals such as iron and copper produce oxidative stress and ROS-induced injury in cells. Some reactions that lead to free radical formation and involved in free radical attacks on lipids are shown in Fig: 3 (Lillian, 1995; McCord, 1998).
Enzymatic free radical formation

\[
\begin{align*}
\text{Enzymatic free radical formation} \\
\text{Xanthine} + O_2 + H_2O & \xrightarrow{\text{Xanthine oxidase}} \text{urate} + O_2^- + 2H^+ \\
\text{NADPH} + 2O_2 & \xrightarrow{\text{NADPH oxidase}} \text{NADP}^+ + 2O_2^- + H^+ \\
\text{NADPH} + \text{2 quinone} & \xrightarrow{\text{Cytocrome P450 reductase}} \text{NADP}^+ + 2\text{semiquinone}^- + H^+
\end{align*}
\]

Nonenzymatic free radical formation

\[
\begin{align*}
\text{Fe}^2+ + H_2O_2 & \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^- \\
\text{Fe}^2+ + O_2 & \rightarrow \text{Fe}^{3+} + O_2^{--} \\
\text{Lipid oxidation by radical attack (L = lipid)} \\
\text{LH} + \text{OH}^- & \rightarrow \text{L}^- + \text{H}_2\text{O} \\
\text{L}^- + O_2 & \rightarrow \text{LOO}^- \\
\text{LOO}^- + \text{LH} & \rightarrow \text{LOOH} + \text{L}^- \rightarrow \text{Chain reaction}
\end{align*}
\]

Fig: 3  Formation of free radicals

Hydroxyl free radical (OH\(^{-}\)) is generated by Fenton reaction from peroxides, is able to abstract a hydrogen atom (H\(^{-}\)) from a wide range of organic compounds, generating a carbon-centered free radical in the process:

\[
\text{R-H} + \text{OH}^- \rightarrow \text{R}^* + \text{H}_2\text{O}
\]

The carbon-centered free radicals (L\(^{*}\)), derived from reaction of (OH\(^{-}\)) with lipid molecules, reacts with oxygen to form lipid peroxy radicals, which initiate a chain reaction with other lipid molecules. The lipid peroxidation process can be terminated by the reaction of two radicals with one another:

\[
\begin{align*}
\text{L}^* + \text{L}^* & \rightarrow \text{L-L} \\
\text{LOO}^* + \text{L}^* & \rightarrow \text{LOOL} \\
\text{LOO}^* + \text{LOO}^* & \rightarrow \text{LOOL} + \text{O}_2
\end{align*}
\]

The lipid peroxy radicals can also undergo intramolecular rearrangement to produce endoperoxides that can transformed into endoperoxy hydroperoxides after further rearrangement and reaction with oxygen. Final product decomposes in the presence of metal ions to produce malondialdehyde and other low-molecular-mass fragments.

Continuous interaction of the animal physiological systems with these free radicals generated either indigenously or from exogenous sources therefore, lead to excess load of free radicals which resulted in a state characterized by a disturbance in the balance between ROS production and ROS removal and
repair of damaged complex molecules on the other, is called oxidative stress (Halliwell, 1999). Therefore, living creatures have evolved a highly complicated defense system with antioxidants composed of enzymes and vitamins against oxidative stress in the course of their evolution. These defense systems are mainly classified as (i) suppression of generation of ROS, (ii) scavenging of ROS, (iii) clearance, repairing and reconstitution of damage and (iv) induction of antioxidant proteins and enzymes. However, amounts of these protective devices present under normal physiological conditions are sufficient only to cope with the normal threshold of physiological rate of free-radical generation. Therefore, any additional burden of free radicals, can tip free radical (pro-oxidant) and anti-free radical (antioxidant) balance leading to oxidative stress. The oxidative stress, defined as the imbalance between oxidants and antioxidants in favour of the former potentially leading to damage has been suggested to be the cause of aging and various human diseases (Tiwari, 2004).

3.4.3 Antioxidant protection:
In biological systems the definition for antioxidants has been extended to any substance that significantly delays or prevents oxidation of the substrate like lipids, proteins, DNA and carbohydrates. Currently however, biological antioxidants have further assumed a broad definition to include repair systems such as iron transport proteins (e.g. transferrin, albumin, ferritin and caeruloplasmin), antioxidant enzymes and factors affecting vascular homeostasis, signal transduction and gene expression.
Antioxidants may exert their effects by different mechanisms, such as suppressing the formation of active species by reducing hydroperoxides (ROO\(^*\)) and \(\text{H}_2\text{O}_2\) and also by sequestering metal ions, scavenging active free radicals, repairing and/or clearing damage and inducing biosynthesis of other antioxidants or defense enzymes (Tiwari, 2004).
Antioxidants act as catalysts in a biomedical reaction, which means they react to oxygen free radicals but are not damaged or changed by them. Such a reaction results in a stable molecule and the unchanged antioxidant is ready to continue its helpful work in transforming oxygen free radicals into harmless molecules (Merrily, 2003).
Antioxidants of biological/therapeutic importance should have the property that they will react/ trap the free-radical before it reacts with the susceptible substrate and initiate chain reaction. Based on several theoretical models and complex calculations, reports concluded that bond dissociation enthalpy (BDE) gives excellent correlation for this requirement with many known families of antioxidants that have been extensively studied in biological systems, like vitamins E and C, resveratrol, gallicatechins, ubiquinol, etc. It was reported that lower the BDE, the more reactive the antioxidant. However, it should not be too low to reduce the molecular oxygen, forming HO$_2^*$ the process of autoxidation.

Based on BDE calculations, a design window proposed for an ideal antioxidant is in the range of 68–76 kcal mol$^{-1}$, i.e. higher than vitamin C and lower than a-tocopherol. Based on these considerations, it has been made possible to design several antioxidant molecules that have displayed excellent biological activities. Simultaneously, it was also observed that majority of antioxidants originating from natural products fall under this criterion (Wright, 2003). Antioxidant protection system involves a variety of components, both endogenous and exogenous which are reported in Fig: 4 (Jacob, 1995; Mark, 1998).

<table>
<thead>
<tr>
<th><strong>Endogenous Antioxidants</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Bilirubin</td>
</tr>
<tr>
<td>- Thiols, e.g., glutathione, lipoic acid, N-acetyl cysteine</td>
</tr>
<tr>
<td>- NADPH and NADH</td>
</tr>
<tr>
<td>- Ubiquinone (coenzyme Q10)</td>
</tr>
<tr>
<td>- Uric acid</td>
</tr>
<tr>
<td>- Enzymes:</td>
</tr>
<tr>
<td>- copper/zinc and manganese-dependent superoxide dismutase (SOD)</td>
</tr>
<tr>
<td>- iron-dependent catalase</td>
</tr>
<tr>
<td>- selenium-dependent glutathione peroxidase</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Dietary Antioxidants</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Vitamin C</td>
</tr>
<tr>
<td>- Vitamin E</td>
</tr>
<tr>
<td>- Beta carotene and other carotenoids and oxycarotenoids, e.g., lycopene and lutein</td>
</tr>
<tr>
<td>- Polyphenols, e.g., flavonoids, flavones, flavonols, and proanthocyanidins</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Metal Binding Proteins</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Albumin (copper)</td>
</tr>
<tr>
<td>- Ceruloplasmin (copper)</td>
</tr>
<tr>
<td>- Metallothionein (copper)</td>
</tr>
<tr>
<td>- Ferritin (iron)</td>
</tr>
<tr>
<td>- Myoglobin (iron)</td>
</tr>
<tr>
<td>- Transferrin (iron)</td>
</tr>
</tbody>
</table>

Fig: 4 Antioxidant protection systems
The antioxidants differ in their affinity to the various radicals as well as in their binding site in the organism. The master of all is vitamin E, but on the surface of biological membranes and of the blood lipids only, whereas in the fluid spaces vitamin C is the main one, as glutathione acts intracellular and melatonin in the nerve cells. Thus antioxidants can be classified according to their radical affinity and tissue concentrations. Various ROS and its neutralizing antioxidants are presented in Fig: 5 (Matkovics, 2001).

<table>
<thead>
<tr>
<th>ROS</th>
<th>Neutralizing Antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl radical</td>
<td>vitamin C, glutathione, flavonoids, lipoic acid</td>
</tr>
<tr>
<td>Superoxide radical</td>
<td>vitamin C, glutathione, flavonoids, SOD</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>vitamin C, glutathione, beta carotene, vitamin E, CoQ10, flavonoids, lipoic acid</td>
</tr>
<tr>
<td>Lipid peroxides</td>
<td>beta carotene, vitamin E, ubiquinone, flavonoids, glutathione peroxidase</td>
</tr>
</tbody>
</table>

Fig: 5 Various ROS and corresponding neutralizing antioxidant systems

3.4.4 Dietary antioxidants

α- tocopherol

The most active component of vitamin E, lipid-soluble, chain-breaking antioxidant by reacting with ROO' before it can attack lipid molecules and protects membrane fatty acids from lipid peroxidation at least the part of this activity is the result of its ability to complex free fatty acids (Fryer, 1992; Mark, 1998). The association between the carboxyl group of the fatty acid and the ring of α- tocopherol reduces this destabilization. The tocopheroxyl radical is stabilized by the fully substituted benzoquinone ring and therefore does not propagate the radical reaction. As the active oxygen of α- tocopherol is located near the surface of the bilayer so that it readily diffuses in bilayer and can't react with peroxyl radicals, formed in the bilayer. This position also allows the tocopheroxyl radical to be reduced by ascorbate in the aqueous phase to regenerate α- tocopherol (Sies and Murphy, 1991).
Ascorbic acid

Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids, which is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated and being capable of regenerating and repairing vitamin E (Sies, 1992; Tiwari, 1999). By reacting with activated oxygen more readily than any other aqueous component, ascorbate protects critical macromolecules from oxidative damage (Asada, 1992).

It will react with superoxide, hydrogen peroxide or the tocopheroxyl radical to form monodehydroascorbic acid and/or dehydroascorbic acid.

$$2 \text{O}_2^{-} + 2 \text{H}^{+} + \text{ascorbate} = 2\text{H}_2\text{O}_2 + \text{dehydroascorbate}$$

$$\text{H}_2\text{O}_2 + 2 \text{ascorbate} = 2\text{H}_2\text{O} + 2 \text{monodehydroascorbate}$$

Tocopheroxyl radical + ascorbate = tocopherol + monodehydroascorbate

The monodehydroascorbate can either spontaneously dismutate or is reduced to ascorbate by NAD (P)H monodehydroascorbate reductase (Asada, 1992).

$$2\text{monodehydroascorbate} = \text{ascorbate} + \text{dehydroascorbate}$$

Monodehydroascorbate + NAD (P)H = ascorbate + NAD (P)

The dehydroascorbate is unstable at pH greater than 6, decomposing into tartrate and oxalate. To prevent this, dehydroascorbate is rapidly reduced to ascorbate by dehydroascorbate reductase using reducing equivalents from glutathione (GSH) (Meister, 1992).

$$2 \text{GSH} + \text{dehydroascorbate} = \text{GSSG} + \text{ascorbate}$$

Carotenoids

β-carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. They are a group of red, orange and yellow pigments found in plant foods and some dietary carotenoids include β-carotene, α-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin. Research suggested that β-carotene may work synergistically with vitamin E (Jacob, 1995; Sies and Stahl, 1995).

Phytonutrients

Other dietary food compounds, such as the phytochemicals in plants and zoo chemicals from animal products, are believed to have greater antioxidant effects than either vitamins or minerals. These are called the non-nutrient antioxidants that include many plant-derived substances collectively termed “phytonutrients,” or “phytochemicals,” and becoming increasingly known for
their antioxidant activity. Phenolic compounds such as flavonoids, in plant kingdom serve as protectors against a wide variety of environmental stresses while, in humans, it appear to function as “biological response modifiers (Briviba and Sies, 1994). Flavonoids have been demonstrated to have hepatoprotective, anti-inflammatory, antiallergenic, anti-viral, anti-aging and anti-carcinogenic activity which can be largely attributed to their antioxidant properties (Kuhnau, 1976; Havsteen, 1983; Middleton, 1984; Cody, 1986).

Many studies have been performed to identify antioxidant compounds with pharmacologically activity and a limited toxicity. In this context, ethnopharmacology represents the most important way possible of finding interesting and therapeutically helpful molecules. The phytochemical analysis of various traditional drugs has revealed a large number of compounds including hydroxyl cinnamic acids, flavonoids, tocopherol, curcumin, ascorbate, carotenoids, polyphenols, etc. which have been shown to have potent antioxidant properties (Gutteridge, 1994; Saija et al., 1995; Van den Berg, 1996).

### 3.4.5 Endogenous antioxidants

Alpha lipoic acid, coenzyme Q₁₀ (CoQ₁₀) and glutathione are produced by the body at the cellular level. Vitamin E and CoQ₁₀ are fat-soluble and protect cell membranes. Vitamin C and glutathione are water-soluble and protect the cytoplasm within cells. Each antioxidant is completely dependent on the others, having its own particular function and the inability to perform the function of another (Merrily, 2003).

**Alpha lipoic acid**

Alpha lipoic acid is an oxygen free radical scavenger and “thiol” or “biothiol,” containing molecule known for its involvement in the reaction that catalyzes the oxidative decarboxylation of α-keto acids, such as pyruvate and α-ketoglutarate. Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), are capable of quenching free radicals in both lipid and aqueous domains and as such has been called a “universal antioxidant. Lipoic acid may also exert its antioxidant effect by chelating with pro-oxidant metals and by sparing effect on other antioxidants (Kagen, 1992; Packer and Witt, 1995).
Glutathione

An important water-soluble antioxidant directly quenches lipid peroxides and vital to proper liver function. When an individual is exposed to high levels of xenobiotics, more glutathione is utilized for conjugation (a key step in the body’s detoxification process) making it less available to serve as an antioxidant (Merrily, 2003, Derick et al., 2006).

Ubiquinone (coenzyme $Q/Q_{10}$)

Ubiquinone exerts main natural function in mitochondria as a part of the electron transport chain, but is also present in low concentrations in plasma and in cell membranes where it functions as an antioxidant by preventing lipid peroxidation (Ernster and Dallner, 1995).

Protective enzymes

Various defense mechanisms are complementary to one another because they act on different oxidants or in different cellular compartments. One important line of defense is a system of enzymes, including glutathione peroxidases, superoxide dismutases and catalase, which decrease the concentration of the most harmful oxidants. Fig: 6 show some of the actions of these enzymes (Lillian, 1995).

Superoxide dismutase

Superoxide dismutases are a family of antioxidant enzymes important in the rapid removal of superoxide radicals by catalytic decomposition to hydrogen peroxide and oxygen. It was thought to be a copper storage protein and was also known as erythrocuprein, indophenol oxidase and tetrazolium oxidase (McCord and Fridovich, 1969). SOD is present in all aerobic organisms and most (if not all) sub-cellular compartments, that generate activated oxygen and has a central role to control oxidative stress (Beyer et al., 1991; Bowler et al., 1992).
Four classes of SODs are known, distinguished by the metal prosthetic group: Cu/Zn, Fe, Mn and Ni. Fe- and Mn-SODs constitute a structural family (Parker et al., 1987; Parker and Blake, 1988). Fe- and Mn-SODs are unequally distributed and located in different cellular compartments. A Cu/Zn SOD is present in the cytosol and in the space between the two membranes surrounding the mitochondria. While, a Mn-SOD is present in the mitochondrial matrix (Fridovich, 1997). The eukaryotic cytosolic Cu, Zn-SOD and the mitochondrial Mn-SOD are now joined by an extracellular Cu, Zn-SOD, which is referred to as EC-SOD. The human enzyme is a homotetrameric protein and it shows some sequence homology to the cytosolic Cu, Zn-SOD. It is glycosylated and exhibits affinity for sulfated polysaccharides, such as heparin or heparan sulfate. Thus, although detectable in blood plasma, most of it exists bound onto the extracellular matrix (Marklund, 1982; Sandstrom et al., 1992; Oury et al., 1995).

**Catalase**

Catalase is an iron-containing enzyme that serves to detoxify hydrogen peroxide and various other molecules and found primarily in the small membrane-enclosed cell components called peroxisomes. Catalase eliminates hydrogen peroxide by catalyzing a reaction between two hydrogen peroxide molecules, resulting in the formation of water and $O_2$. In addition, it can promote the interaction of hydrogen peroxide with compounds that can serve as hydrogen donors so that the hydrogen peroxide can be converted to one molecule of water and the reduced donor becomes oxidized (a process sometimes called the peroxidatic activity of catalase). Compounds that can provide these hydrogen atoms include beverage alcohol. Catalase also detoxifies different substrates like phenols and alcohols, via coupled reduction of hydrogen peroxide. It lowers the risk of hydroxyl radical formation from $H_2O_2$ via the Fenton- reaction catalyzed by Cu or Fe ions (Fridovich, 1999; Halliwell, 1999).

**Peroxiredoxins (Prx)**

Peroxiredoxins are recently discovered enzymes capable of directly reducing peroxides, e.g., hydrogen peroxide and different alkyl hydroperoxides (Kim et al., 1988). In the mitochondria of mammalian cells the mitochondrial thioredoxin system is probably a specific reductant of Prx (Miranda et al.,
2000). Peroxiredoxins have been shown to inhibit apoptosis induced by p53 (Zhou et al., 2000) and by hydrogen peroxide (Zhang et al., 1997). As of today, at least 13 mammalian peroxiredoxins are known (Butterfield et al., 1999; Chae et al., 1999; Chae et al., 1999a).

**Glutathione peroxides (GPx)**

This system consists of enzymes, glutathione peroxidase, glutathione reductase and the cofactors glutathione (GSH) and reduced NADPH. These molecules effectively remove hydrogen peroxide. GSH consists of three amino acids, is an essential component of this system and serves as a cofactor for an enzyme called glutathione transferase, which helps remove certain drugs and chemicals as well as other reactive molecules from the cells (Defeng et al., 2003).

There are at least 4 different GPx in mammals (GPx1—4), all of them containing selenocysteine. GPx1 and GPx4 are both cytosolic enzymes abundant in most tissues while GPx2 (gastrointestinal GPx) and GPx3 (plasma GPx) are mainly expressed in the gastrointestinal tract and kidney, respectively (Dreher et al., 1997; Mates et al., 1999).

\[ \text{ROOH} + 2\text{GSH} \rightarrow \text{ROH} + \text{GSSG} + \text{H}_2\text{O} \]

The catalytic mechanism (Epp et al., 1983) involves oxidation of the active site selenolate (Se) to selenenic acid (SeOH). Upon addition of one molecule of GSH, the selenenic acid is transformed to a selenenylsulfide adduct with glutathione (Se-SG), which can be regenerated to the active selenolate and glutathione disulfide (GSSG) by addition of a second molecule of GSH. Thus, in the reaction, two molecules of GSH are oxidized to GSSG that subsequently can be reduced by glutathione reductase under consumption of NADPH. Some data has indicated that GPx should be of high antioxidant importance under physiological conditions (Jones et al., 1981) while others place the enzymes as important only at events of oxidative stress (Kelner and Bagnell, 1990).

**Other glutathione-related systems**

Glutathione functions as a sulfhydryl buffer and also serves to detoxify compounds either via conjugation reactions catalyzed by glutathione S-transferases (Armstrong, 1997; Van Bladeren, 2000) or directly, as is the case with hydrogen peroxide in the GPx catalyzed reaction (Williams, 1992).
Another class of proteins related to GSH is the glutaredoxins (Grx), with functions overlapping those of thioredoxins. A major qualitative difference between Grx and Trx is that Grx can be reduced by GSH and is capable of reducing GSH mixed protein disulfides formed at oxidative stress, which should play an important role in the total cellular antioxidant defense (Holmgren, 2000).

**Thioredoxin System**

Thioredoxin reductase (TrxR) in conjunction with thioredoxin (Trx) is an oxidoreductase system with antioxidant and redox regulatory roles. Mammalian TrxR has a highly reactive active site selenocysteine residue resulting in a profound reductive capacity, reducing several substrates in addition to Trx. The properties of TrxR in combination with the functions of Trx position this system at the core of cellular thiol redox control and antioxidant defense (Jonas and Elias, 2001). Thioredoxins are proteins with three distinct variants of human Trx encoded by separate genes have been cloned and characterized. Most studied is the gene for Trx-1 (Taniguchi et al., 1996) while Trx-2 includes a 60 amino acid (Spyrou et al., 1997). The third, SpTrx, is expressed in spermatozoa (Miranda et al., 2000).

**Thioredoxin reductase**

Thioredoxin reductase (TrxR) isoenzymes are oxidoreductases that reduce the active site disulfide in oxidized Trx (Holmgren, 1989; Holmgren and Bjornstedt, 1995; Gromer et al., 1999; Arner and Holmgren, 2000). Mammalian TrxR reduces not only the disulfide in oxidized Trx, but also some other protein disulfides or a wide spectrum of oxidized low molecular weight compounds (Arner et al., 1999). It is closely related to other mammalian oxidoreductases such as glutathione reductase (Sandalova et al., 2001) and general features in the catalytic mechanism (Arscott et al., 1997) but different in a C-terminal elongation (Zhong et al., 1998) which explains many of the unique features of this enzyme.
3.5 Oxidative stress and liver disorders:

Oxidative stress plays an important role in the pathogenesis of toxic liver diseases and of other hepatic alterations. Various animal experiments and human clinicopharmacological studies revealed that drugs belong in the group of free-radical scavengers, their mechanism of action involving membrane stabilization, neutralization of free radicals and immunomodulation (Feher et al., 1998).

Oxidative stress and hepatic mitochondria play major role in the pathogenesis of nonalcoholic fatty liver disease. Nonalcoholic fatty liver disease (NAFLD) involves fatty liver (hepatic steatosis) and nonalcoholic steatohepatitis (NASH) that progresses from hepatic steatosis with lobular inflammation to ballooning degeneration, fibrosis and eventually to cirrhosis (Matteoni et al., 1999; Falck et al., 2001). NAFLD is associated with several predisposing factors such as obesity, diabetes, dyslipidemia, jejunoileal bypass, drugs and parenteral nutrition. Many studies have shown that liver injury is mediated by oxidative stress (Chitturi and Farrell, 2001; Mehta et al., 2002), endotoxins, cytokines (Tilg and Diehl, 2000; Wigg et al., 2001) and hyperinsulinemia (Luyckx et al., 2000; Scheen and Luyckx, 2003). Oxidative stress plays a central role in the pathogenesis of NASH. The increased production of reactive oxygen species (ROS) is known to cause lipid peroxidation, followed by an inflammatory response and activation of stellate cells leading to fibrogenesis (Curzio et al., 1985; Lee et al., 1995; Yang et al., 2000). Various reports on the role of liver mitochondrial dysfunction and oxidative stress in the pathogenesis of experimental fatty liver suggested that lipid peroxidation products can impair the flow of electrons along the respiratory chain, causing over reduction of respiratory chain components and enhanced mitochondrial reactive oxygen species. Mitochondria are responsible for oxidative phosphorylation and fatty acid β-oxidation and are the main source of cellular ROS. Therefore, dysfunction of liver mitochondria may play an important role in the induction of hepatic steatosis and NASH. Under normal conditions, hepatic aerobic metabolism involves a steady-state production of pro-oxidants such as reactive oxygen species and reactive nitrogen species (RNS), which are balanced by a similar rate of their consumption by antioxidants. Imbalance
in the pro-oxidant/antioxidant equilibrium in favour of pro-oxidants constitutes the oxidative stress phenomenon, a condition that may induce a number of pathophysiological events in the liver. Hepatotoxicity by oxidative stress may be achieved through a direct attack of ROS and RNS on essential biomolecules with loss of their biological functions and cell viability (Sies, 1986; Videla et al., 1995; Kaplowitz, 2000). Alternatively, ROS may indirectly activate redox sensitive transcription factors such as nuclear factor κB (NF-κB) (Baueuerle and Henkel, 1994) or activator protein-1 (AP-1) (Karin et al., 1997), thus triggering the production of cytotoxic, proinflammatory and/or fibrogenic mediators by Kupffer cells and other non-parenchymal cells (Tilg and Diehl, 2000).

Mitochondrial dysfunction plays central role in the accumulation of fat in the liver ("first hit") and the excessive production of ROS results in lipid peroxidation ("second hit") (Wigg et al., 2001). This increased mitochondrial ROS formation may further oxidize fat deposits, causing a vicious cycle with more lipid peroxidation, more mitochondrial damage and more ROS formation. ROS may oxidize fat deposits, releasing lipid peroxidation products that damage mitochondrial DNA and proteins to partially block the flow of electrons along the respiratory chain, thus further increasing mitochondrial ROS formation. ROS may also deplete antioxidants and cause the formation of tumor necrosis factor-α, two effects that may further impair the flow of electrons and increase mitochondrial ROS formation (Pessayre et al., 2002).

It is well known that a substantial increase in steatosis and fibrosis usually leads to potentially lethal cirrhosis of the liver in humans. The high global prevalence of these hepatopathies places them among the most serious diseases. Although the pathogenesis of liver fibrosis is not quite clear, there is no doubt that reactive oxygen species play an important role in pathological changes in the liver, particularly in cases of alcoholic and toxic liver diseases (Poli and Parola, 1997).

Alcohol liver disease is a major health problem worldwide (Befrity et al., 1995). One aspect of alcohol toxicity that has received increasing attention in recent years is the role of free radical species in the etiology of liver injury (Normann et al., 1992). It is not unlikely that hepatic toxicity due to ethanol is probably multifactorial; nonetheless, evidence for the role of free radicals in
the pathogenesis of liver disease has been reported (Lecomte et al., 1994). Animal experimentation has demonstrated that either acute or chronic alcohol administration increases the rate of lipid peroxidation (Diehi et al., 1988), which is a classical biochemical feature in hepatotoxic poisoning (Dianzani et al., 1991; Lieber, 1993). A self-propagating chain of free radicals absorbs electrons from cell membrane to attain electrochemical stability (Stohs, 1995; Levine and Kidd, 1985). This alters the integrity cell membranes. Biological antioxidants prevent free radical oxidative processes by free radical energy reduction, interruption of its chain reaction and prevention of its formation (Traber, 1999).

Biological membranes are particularly prone to the ROS effect. The peroxidation of unsaturated fatty acids in biological membranes leads to a decrease of membrane fluidity and to a disruption of membrane integrity and function, which is implicated in serious pathological changes (Halliwell, 1987). Several endogenous protective mechanisms have been evolved to limit ROS and the damage caused by them (Sies, 1993). However, since this protection may not be complete, or when the formation of ROS is excessive, additional protective mechanisms of dietary antioxidants may be of a great importance. Therefore, many natural and artificial agents possessing antioxidative properties have been proposed to prevent and treat hepatopathies induced by oxidative stress (Lieber, 1997; Eervinkova and Drahota, 1998). There is increasing evidence for the hepatoprotective role of hydroxy- and polyhydroxy-organic compounds particularly from vegetables, fruits and some herbs (Bass, 1999). Especially in humans, different kinds of tea are amongst the most popular non-alcoholic beverages that contain a wide range of various natural antioxidants (Cao et al., 1996; Dreosti, 1996; Kohlmeier et al., 1997).

**Conventional Medicines for Liver Disorders:**

Immunoglobulin (Ig) is quite effective against hepatitis A when administered to anyone exposed to the virus as soon as possible or within two weeks after jaundice appears. Vaccines for hepatitis are now a common feature of immunization programs the world over. Chronic cases of hepatitis B and C are treated with interferon.

In the treatment of cirrhosis diuretics, vitamins and abstinence from alcohol are supportive measures with rest and nutritional meals. Liver abscesses are treated
with long-term administration of antibiotics such as amino glycosides, cephalosporins, clindamycin, or chloramphenicol. If *E. coli* is the cause of infection, treatment includes ampicillin. For *E. histolytica*, chloroquine (aralen) or metronidazole (flazyl) is included. Vitamin B₆ and d-penicillamine as well as corticosteroids such as prednisone are administered in cases of Wilson's disease (Owais et al., 2006).

Despite advancements in modern medicine, no hepatoprotective medicine is available. Treatment options for cirrhosis, fatty liver and chronic hepatitis are limited as well as problematic. The conventional drugs used in such treatments are corticosteroids, interferon, colchicines, penicillamine, antiviral and immune suppressant drugs which may themselves cause damage (e.g. azathioprine can cause cholestatic jaundice), while interferons and virazole can cause elevation of serum transaminase (Cattral et al., 1999; Fujimori et al., 2002). Alternative treatments for liver diseases to replace the currently used drugs need to be given impetus in the light of current findings from research studies and publications in the field of herbal treatment of liver diseases, especially during the last quarter of the twentieth century.

**Herbal medicines - potential therapeutic agents with minimal side effects.**

Botanical medicines have been used traditionally worldwide for the prevention and treatment of liver disease. Clinical research has confirmed the efficacy of several plants in the treatment of liver disease, while basic scientific research has uncovered the mechanisms by which some plants provide their therapeutic effects. With lack of safe and effective treatment for liver diseases, alternative therapies that curb symptoms with minimum adverse effects on patients are considered more reliable. Several hundred plants have been examined for use in a wide variety of liver disorders but only handfuls have been fairly well researched. These plants include *Silybum marianum* or *Carduus marianum* (milk thistle), *Picrorhiza kurroa* (kutkin), *Curcuma longa* (turmeric), *Camellia sinensis* (green tea), *Rosemarinus officinalis*, *Siberian Ginseng Root*, *Ginkgo biloba*, *Schizandra chinensis*, *Schizandra chinensis* and *Glycyrrhiza glabra* (licorice).

Several hundred other plants are reported to have hepatoprotective properties (18) and a number of studies have been conducted taking into consideration

Medicinal plants are used alone or in different combinations in the preparation of around three dozen patented herbal formulations and number of plants has been studied for their antihepatotoxic potential. However in most cases, the mechanism of their hepatoprotective effect still remains to be ascertained. Most of the plants have been shown to stimulate secretion of bile fluid (choleretic) and salt (chologogue) in experimental animals (Handa et al., 1986).

In India, about 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity (Handa et al., 1986). Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthines. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of about 25 plants have been reported to cure liver disorders (Sharma et al., 2002). Many formulations containing herbal extracts are sold in the Indian market for liver disorders. But management of liver disorders by a simple and precise herbal drug is still an intriguing problem. Several Indian
medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorder. Some of these plants have already been reported to possess strong antioxidant activity (Aniya et al., 2002; Achuthan et al., 2003; Gupta et al., 2006).

Some of the constituents isolated from these hepatoprotective plants and reported to have antihepatotoxic activity include kaempferol, caffeic acid, ferulic acid and p-coumaric acid (Capparis spinosa), azelaic acid, α-amyrin, bornyl acetate, β-sitosterol, daucosterol (Cichorium intybus), (Scott, 1999) nigrumnins I and II (Solanum nigrum) (Ikeda et al., 2000), arjunetoside, oleanolic acid, arjunic acid and arjunaphthanoloside (Terminalia arjuna), andrographolide (Andrographis paniculata), silybin and silymarin (Silybum marianum), kutkoside and picroside I and II (Picrohiza kurroa), gomishins (Schizandra achinensis), schisandrin A (Schizandra chinensis), glycyrrhizin and glycyrrhizinic acid (Glycyrrhiza glabra), saikosaponins (Bupleurum falcatum), sarmantosins (Sedum sannentosum), catechin (Anacardium occidentalis), ursolic acid (Eucalyptus spp.) (Saraswat et al., 1996; Shukla et al., 1996), curcumin (Curcuma longa) and fumaric acid (Sida cordifolia) (Chrungo et al., 1997).

Although some herbal medicines are effective in the treatment of diseases; against which modern medicines are inefficient, very often these drugs are unscientifically exploited and improperly used. Numerous plants and polyherbal formulations are used for the treatment of liver diseases. However, in most of the severe cases, the treatments are not satisfactory. Experimental evaluation in most cases has been incomplete and insufficient and the therapeutic values have been tested against chemically induced subclinical levels of damage in rodents. Even common dietary antioxidant and micronutrients such as tocopherol (Bendich et al., 1986), ascorbic acid, β-carotene (Kunert et al., 1983), glutathione, uric acid and bilirubin and proteins such as caeruloplasmin can provide protection from liver damage.

The synergistic action of various ingredients of a polyherbal formulation for holistic and long-lasting cure of hepatic disorder might help in regulating the metabolism, which is one of the factors responsible for longevity. Various experimental and clinical studies by different researchers have been well documented in this subject field. p-methoxy benzoic acid isolated from
Capparis spinosa was found to possess potent hepatoprotective activity against CC14, paracetamol (in vivo) and thiacetamide galactosamine- (in vitro) induced hepatotoxicity (Gadgoli et al., 1999).

The hepatoprotective effect of Cichorium intybus was observed against CC14-induced hepatotoxicity (confirmed by histopathological examination) and reported to provide significant prevention of the elevation of malondialdehyde formation (plasma and hepatic) and also evaluated using other biochemical parameters (Zafar et al., 1998; Aktay et al., 2000; Ahmed et al., 2003).

All these studies suggest that the observed hepatoprotective effects might be due to the ability to suppress the oxidative degradation of DNA in the tissue debris (Sultana et al., 1995).